The Transforming *Streptococcus Pneumoniae* in the 21st Century

Yu-Chia Hsieh, MD, PhD; Wen-Sen Lee, MD; Pei-Lan Shao, MD; Luan-Yin Chang, MD, PhD; Li-Min Huang, MD, PhD

*Streptococcus pneumoniae*, an important pathogen causing sepsis, sinusitis, otitis media, bacterial meningitis and bacterial pneumonia, results in global morbidity and mortality each year. The burden of pneumococcal disease is highest in children and the elderly. Treatment of pneumococcal infection has been hampered by the complexity of the host immune response. In recent decades, the increase of *S. pneumoniae* strains' resistance to β-lactam antibiotics and other classes of antimicrobials has made treatment even more complicated. Fortunately, the advent of heptavalent conjugate vaccine confers a high degree of protection against pneumococcal disease and colonization caused by vaccine serotype strains. After the introduction of conjugate pneumococcal vaccine, invasive pneumococcal disease caused by vaccine serotypes and antibiotic-resistant isolates has been reduced. However, naturally transformable pneumococci may escape vaccine-induced immunity by switching their capsular genes to non-vaccine serotypes. Development of cheaper, serotype-independent vaccines based on a combination of protein antigens should be pursued. *(Chang Gung Med J 2008;31:117-24)*

Key words: *Streptococcus pneumoniae*, conjugate vaccine, transformable

*Streptococcus pneumoniae*, a pathogen discovered more than one hundred years ago, remains a leading cause of bacteremia, sinusitis, otitis media, bacterial meningitis and pneumonia. This bacterium is present worldwide, and is associated with substantial illnesses and deaths in humans. Historically, study of the biology of *S. pneumoniae* led to the identification of the nature of genetic material, the phenomenon of quorum sensing, the use of polysaccharide-based vaccine and the recognition of bacterial resistance to antimicrobial drugs. Since the complete genome of *S. pneumoniae* was decoded in 1997, much has been discovered about the bacterial proteins involved in pneumococcal disease, the regulation of virulence and the regulation of DNA uptake. Recently, the landscape of pneumococcal infection has been changed by two major events, namely, availability of conjugate pneumococcal vaccine and more aggressive behavior of pneumococcal pneumonia. It is now a good time to review our under-
standing of the biology and clinical behavior of *S. pneumoniae*.

**S. pneumoniae virulence factors**

**Capsule**

Polysaccharide capsule is the earliest known *S. pneumoniae* virulence factor, and serves as a paradigm for studies of immune responses and polysaccharide biochemistry. Capsular polysaccharide is composed of multiple sugars that help pneumococci fight against phagocytosis. The amount of capsule expression in the microbe changes during replication in the host, a phenomenon known as phase variation. Reduced capsule expression (transparent variant) in the nasopharynx is instrumental in exposing the adhesins necessary for colonization, whereas increase in capsule expression (opaque variant) is essential for avoiding complement-mediated opsonophagocytosis during invasive disease. Several factors such as BOX elements, capsule locus A (CpsA), CpsB, CpsC and CpsD, and spontaneous sequence duplication contribute to the complex regulation of capsule synthesis.

**Choline-binding proteins**

*S. pneumoniae* possesses several choline-binding proteins on its surface that serve as a way of attaching it to the cell surface. The most well-known choline-binding proteins in pneumococci are autolysin, pneumococcal surface protein C (PspC) and pneumococcal surface protein A (PspA). Autolysin (LytA amidase) degrades peptidoglycan of the pneumococcal cell wall and separates daughter cells. Lysis of pneumococci by autolysin leads to release of the pneumococcal cell wall and pneumolysin, which in turn induce inflammatory responses and cause tissue damage. PspA is a protective antigen of *S. pneumoniae*, and is able to inhibit complement deposition and activation. It contributes to pneumococcal virulence in both bacteremia and sepsis models. PspC, also referred to as choline-binding protein A (CbpA), acts as an adhesin, and interacts with the polymeric immunoglobulin receptor (pIgR) on mucosal epithelial cells to facilitate adhesion and invasion.

**Pneumolysin and other virulence factors**

The role of pneumolysin in pneumococcal infection has been well studied. Pneumolysin belongs to the family of pore-forming toxins, which can lyse cell membranes containing cholesterol. This toxin also activates the complement system, induces the production of proinflammatory mediators, recruits inflammatory cells and causes cell apoptosis. Other proteins, including LPXTG-anchored protein (hyaluronidase, neuraminidase and serine protease), lipoprotein, hydrogen peroxide, superoxide dismutase, NADH (nicotinamide adenine dinucleotide, reduced form) oxidase, as well as zinc metalloprotease (immunoglobulin A protease, ZmpB and ZmpC), also contribute to the virulence of *S. pneumoniae*. A pneumococcal pilus encoded by the rlrA pathogenicity islet, consisting of LPXTG-containing surface proteins and sortases, enhances adherence and stimulates the host inflammatory response. Pneumococcal neuraminidases cleave sialic acid-containing substrates. Neuraminidase A and B both have essential roles in respiratory tract infection and sepsis. Neuraminidase C may contribute to the ability of pneumococci to cause meningitis.

**Capsular type or clonal type determine the invasive capacity of *S. pneumoniae***

*S. pneumoniae* can be divided into more than 91 distinct types according to capsular polysaccharides but only 20 to 30 types are associated with human diseases. Hence, there is an association between serotype and the potential of pneumococci to cause invasive disease. Certain serotypes, such as serotype 1 are highly invasive, mostly due to the specific chemical composition of their capsules. Serotype 3 can evade the immune system, readily resulting in a fatal disease. Further studies of the population biology of *S. pneumoniae* found that, even within the same serotype, some individual clones (such as ST9 and ST124) were significantly overrepresented in invasive diseases compared with carriage. So far, the exact mechanisms of why some serotypes can go beyond colonization to cause invasive disease remain unclear but it appears that the capsule is not sufficient to determine invasive potential or inflammatory response. The genetic background of the host, in addition to the capsule, also plays a critical role in dictating virulence. Understanding the underlying mechanism of virulent genotypes becomes a priority in the era of the pneumococcal conjugate vaccine.
Innate immunity

*S. pneumoniae* infection is countered by a robust inflammatory reaction in the host. Complement, C-reactive proteins (CRP), surfactant protein, Toll-like receptors (TLR) and T cells comprise the major components of the immune response against *S. pneumoniae*. Studies using mice deficient in specific genes indicated that both the classical and alternative complement pathways were vital in host defense against pneumococcal infection. CRP specifically binds to phosphocholine residues of C-polysaccharide (PnC) in the cell wall of *S. pneumoniae* to activate the classical pathway of complement in human serum. CRP also recognizes pneumococcal lipoteichoic acid (LTA) and cell wall peptidoglycan to initiate an inflammatory response. TLR2 also had a protective role in systemic infection and nasopharyngeal colonization in a murine model. TLR4 recognizes pneumococcal pneumolysin to limit pneumococcal proliferation in the nasopharynx. TLR4 also interacts with pneumolysin to induce mammalian cell apoptosis against pneumococcal infection. CD4 (cluster of differentiation 4) T cells were found to contribute to early protective immunity to *S. pneumoniae* based on studies using mice lacking the major histocompatibility complex II (MHCII) gene. However, how CD4+ T cells function in this aspect remains unclear.

Pneumococcal colonization

The first step leading to pneumococcal disease is nasopharyngeal colonization. *S. pneumoniae* spreads through respiratory droplets. Following exposure, the pathogen may establish itself in the nasopharynx of the new host. The human nasopharynx is the only known natural reservoir for *S. pneumoniae*. Invasive pneumococcal disease occurs when pneumococci gain access into deep human tissues, which might be facilitated by prior virus infection, especially influenza virus infection. *S. pneumoniae* invades human nasopharyngeal epithelial cells through a process termed reverse endocytosis mediated by pIgR. Nasopharyngeal colonization is dynamic, and influenced by overcrowding, smoking, ethnicity and socioeconomic status.

Evolution of *S. pneumoniae*

*S. pneumoniae* was the first pathogen to demonstrate the phenomenon of transformation. In 1944, Avery et al. proved that the genetic material in bacterial cells was DNA by using a transformation model in *S. pneumoniae*. Natural competence for genetic transformation in *S. pneumoniae* is mediated by a quorum sensing-regulated system. CSP, a heptadecapeptide pheromone, induces competence in growing cells at a critical cell density by activating the 2-component signal transduction system comDE. Due to the ability to undergo horizontal gene transfer, *S. pneumoniae* easily adapts to environmental changes, which leads to substantial genetic heterogeneity as well as genomic plasticity (Fig. 1). The first example is the presence of divergent mosaic blocks in penicillin binding protein (PBP) genes in penicillin-resistant *S. pneumoniae* under the selective pressure of penicillin pressure, evolutionary pressure, and vaccine pressure.
penicillin. Mosaic PBP genes evolve to be penicillin-resistant via acquiring PBP from other *Streptococcus* species.\(^{(43)}\) The second example is the evolution to greater virulence via recombination. Serotype 6B causes more invasive diseases than serotype 6A. By using multilocus sequence typing, serotype 6B clones evolved almost exclusively by recombination, whereas serotype 6A evolved by mutation.\(^{(44)}\) The third example is capsular switching under a large-scale vaccination program.\(^{(45)}\) Although the current introduction of conjugate pneumococcal vaccine has successfully reduced invasive pneumococcal disease caused by the vaccine serotypes and effectively decreased the spread of antimicrobial drug-resistant isolates, pneumococcal infection remains a major issue. At least two consequences have been noted since the use of heptavalent conjugate vaccine. First, serotypes not covered by the conjugate vaccine have increased both in nasopharyngeal colonization and clinical illness.\(^{(45)}\) Second, serotype switching can occur through recombination in naturally transformable clones and result in the acquisition of a non-vaccine capsule to escape vaccine-induced immunity.\(^{(45)}\) Furthermore, the ability of different serotypes to be transformed affected the evolutionary biology and genetic diversity of each serotype. Serotype competence accounts for why the reported serotypes that underwent *in vivo* capsular transformation were also antibiotic-resistant. Gene transfer has been a powerful tool in the evolution of *S. pneumoniae*.

### Emerging disease: complicated pneumonia

*S. pneumoniae* is the most common pathogen of pyogenic pneumonia in children. Previous studies have shown that the lungs return to normal after pneumococcal pneumonia, regardless of the severity at the peak stage of the disease. This is for two reasons. First, *S. pneumoniae* usually induces granulocyte apoptosis, which tends to limit tissue injury and promotes the complete resolution of pneumonia.\(^{(46)}\) Second, *S. pneumoniae* produces few exotoxins capable of inducing lung damage, in contrast to other organisms such as *Staphylococcus aureus* and *Streptococcus pyogenes*, which produce a variety of tissue-damaging substances causing lung necrosis and destructive lung injury.\(^{(47)}\) Since the advent of the use of penicillin, *S. pneumoniae* infection has rarely developed into empyema or lung necrosis. However, an increase of complicated pneumococcal pneumonia, including necrotizing pneumonia, lung abscess and empyema, has been observed in children since the 1990s\(^{(5,48,49)}\) (Table 1). The occurrence of complicated pneumonia was associated with longer durations of fever, longer oxygen requirement and longer hospital stays.\(^{(5,48)}\) Older age, white race, presence of immature polymorphonuclear leukocytes in peripheral blood, high CRP levels, no underlying disease or chest pain on presentation were predictors of lung necrosis and/or abscess.\(^{(5,48)}\) This increase of

#### Table 1. Studies of an Increase in Complicated Pneumococcal Pneumonia

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Year</th>
<th>Pattern of complicated pneumonia</th>
<th>Prevalent serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>(5)</td>
<td>U.S.A.</td>
<td>1993-2000</td>
<td>necrotizing pneumonia, pleural effusion, empyema and lung abscess</td>
<td>14, 1, 19, 6, 3</td>
</tr>
<tr>
<td>(50)</td>
<td>U.S.A.</td>
<td>1996-2000</td>
<td>empyema</td>
<td>1, 14, 6B, 19F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2001-2005</td>
<td>empyema</td>
<td>1, 3, 19A</td>
</tr>
<tr>
<td>(51)</td>
<td>U.K.</td>
<td>1997-2001</td>
<td>empyema</td>
<td>1, 14, 3</td>
</tr>
<tr>
<td>(54)</td>
<td>U.K.</td>
<td>1997-2003</td>
<td>cavitary disease</td>
<td>1, 3, 14, 9V</td>
</tr>
<tr>
<td>(48)</td>
<td>Taiwan</td>
<td>1995-2003</td>
<td>necrotizing pneumonia, empyema</td>
<td>14, 23, 19, 9</td>
</tr>
<tr>
<td>(49)</td>
<td>Israel</td>
<td>1986-1997</td>
<td>pleural effusion, empyema, pneumothorax, pneumatocele and/or aplectasis</td>
<td>Not done</td>
</tr>
<tr>
<td>(55)</td>
<td>Singapore</td>
<td>1997-1999</td>
<td>cavitary necrosis, abscess formation, empyema</td>
<td>Not done</td>
</tr>
<tr>
<td>(56)</td>
<td>Spain</td>
<td>1993-2003</td>
<td>parapneumonic pleural effusion</td>
<td>Not done</td>
</tr>
</tbody>
</table>

**Abbreviation:** PCV: heptavalent conjugate vaccine.
complicated pneumonia is not directly related to the increase in penicillin-resistant \textit{S. pneumoniae}.\textsuperscript{5,48,49} In the U.S., serotype 14 was the most common serotype causing complicated pneumonia, whereas serotype 1 and serotype 3 significantly caused complicated pneumonia compared to those serotypes causing lobar pneumonia in children before the widespread use of heptavalent pneumococcal conjugate vaccine.\textsuperscript{5} After the utilization of conjugate vaccine, serotype 1 remained prevalent, and serotypes 3 and 19A were increasingly detected.\textsuperscript{50} In the U.K., serotype 1 was also the dominant serotype causing pneumococcal empyema.\textsuperscript{51} Clonal spread of pneumococcal serotype 1 is speculated to contribute to the increased complicated pneumonia in the U.S. and U.K. Interestingly, serotype 1 \textit{S. pneumoniae} was rare in the nasopharynx but had a high clinical incidence. This serotype was common in both Northern Europe and North America in the early 20th century, and now has become more prevalent in developing countries such as Rwanda, Egypt and Africa. Poverty, overcrowding and decreased availability of antibiotics all contribute to the spread of serotype 1.\textsuperscript{52} In view of the rare carriage of serotype 1 \textit{S. pneumoniae}, it is mysterious as to how it is transmitted among humans. In most cases of culture-negative parapneumonic pleural effusion or empyema, serotype 1 was the frequent etiology.\textsuperscript{51,53} Surprisingly, several studies failed to identify serotype 1 in clinical samples in Taiwan. Instead, the major clone associated with complicated pneumonia in Taiwan was serotype 14.\textsuperscript{48} Since serotype 1 is difficult to culture, whether there is real serotype difference in complicated pneumonia is worth further study in Taiwan.

\section*{Conclusion}

Given the proclivity of horizontal gene transfer, current advances in antimicrobial therapy and serotype-limited conjugate vaccine are inadequate to combat pneumococcal diseases. In the future, better understanding of molecular interaction at the cellular level could provide insight into the development of protein vaccine and new modulation therapy.

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二十一世紀轉化中的肺炎雙球菌

謝育嘉 李文生 邵蓓嵐^1 張鑾英^1 黃立民^1

肺炎雙球菌是造成敗血症、鼻竇炎、中耳炎、細菌性腦膜炎的重要病因之一，每年都造成相當大的發病率及死亡率。這個疾病特別容易發生在兒童及老年人身上。當宿主受肺炎雙球菌感染時，複雜的免疫反應使得治療效果受限。近幾年來，抗藥性肺炎雙球菌的產生使得肺炎雙球菌的治療更為困難。幸運的是，由於7價結合型肺炎雙球菌疫苗的使用，成功的降低了疫苗型侵襲性肺炎雙球菌疾病的發生，也減少了抗藥性肺炎雙球菌的散播。雖然如此，由於肺炎雙球菌具有自然勝任能力，能夠將原本的疫苗型荚膜轉換成非疫苗型荚膜。宿主由於疫苗的保護，不會被疫苗型肺炎雙球菌感染，但有可能會受到非疫苗型肺炎雙球菌的侵襲。在未來，發展一個更經濟實惠，不受荚膜型限制的蛋白疫苗，將是努力的目標。(長庚醫誌2008;31:117-24)

關鍵詞：肺炎雙球菌，結合型疫苗，轉化的